Antibiotic Resistance Genes in the Human-Impacted Environment : A One Health Perspective

Tiedje, James M.

2019-06

Tiedje , J M , Wang , F , Manaia , C M , Virta , M , Sheng , H , Ma , L , Zhang , T & Topp , E 2019 , ' Antibiotic Resistance Genes in the Human-Impacted Environment : A One Health Perspective ' , Pedosphere , vol. 29 , no. 3 , pp. 273-282 . https://doi.org/10.1016/S1002-0160(18)60062-1

http://hdl.handle.net/10138/329957
https://doi.org/10.1016/S1002-0160(18)60062-1

Antibiotic Resistance Genes in the Human Impacted Environment: a One Health Perspective

James M. Tiedje [a,b], Fang Wang [a,b,c*], Célia M. Manaia [d], Marko Virta [e], Hongjie Sheng [a,b], Liping Ma [f], Tong Zhang [f], Edward Topp [g]

[a] *Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China*

[b] *Center for Microbial Ecology, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, Michigan 48824, United States*

[c] *University of Chinese Academy of Sciences, Beijing 100049, China*

[d] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Arquiteto Lobão Vital, 172, 4200-374 Porto, Portugal*

[e] *Department of Microbiology, University of Helsinki, Viikinkaari 9, 00014 University of Helsinki, Finland*

[f] *Environmental Biotechnology Lab, The University of Hong Kong, Hong Kong, China*

[g] *London Research and Development Centre, Agriculture and Agri-Food Canada, and University of Western Ontario, London, ON, Canada*

*Corresponding author. E-mail: wangfang@issas.ac.cn

Abstract

Antibiotic resistance and its environmental component is gaining more attention as part of combating the growing healthcare crisis. The One-Health framework, promulgated by many global health agencies, recognizes that antimicrobial resistance is a truly inter-domain problem in which human health, animal agriculture and the environment are the core and interrelated components. This prospectus presents the status and issues relevant to the environmental component of antibiotic resistance, namely the needs for advancing surveillance methodology: the environmental reservoirs and sources of resistance, namely urban wastewater treatment plants, aquaculture production systems, soil receiving manures and biosolids and the atmosphere which includes longer range dispersal. Much recent work has been done describing antibiotic resistance genes in various environments; now quantitative, mechanistic and hypothesis driven studies are needed to identify practices that reduce real risk and maintain effectiveness of our current antibiotics as long as possible. Advanced deployable detection methods for antibiotic resistance in diverse environmental samples are needed to provide the surveillance information to identify risk and define barriers that can reduce risk. Also needed are practices that reduce antibiotic use and thereby reduce selection for resistances, as well as practices that limit dispersal or destroy antibiotic resistant bacteria or their resistance genes that are feasible for these varied environmental domains.

Key words: Antibiotic Resistance, Transmission, Agriculture, One-Health

1 Introduction

The overuse and misuse of antibiotics for human therapy and livestock production around the world over the past decades have increased antimicrobial resistance in diverse environments (Berendonk *et al.*, 2015; Garbisu *et al.*, 2018). Exposure to antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment can increase the prevalence of resistance determinants in the human microbiome (Leonard *et al*., 2015). In December 2017, the United Nations Environment Programme identified environmental antibiotic resistance as the top of six emerging issues of concern (UNEP, 2017). Together with climate change, water stress and environmental degradation, widespread antibiotic resistance should be regarded as one of the global challenges humans face in this century.

ARGs have been widely identified in various environments, including wastewater and sludge (Manaia *et al.*, 2018), livestock farms and soil (Zhu *et al.*, 2013), river water and sediment (Muziasari *et al.*, 2017), drinking water (Ma *et al.*, 2017a), glacier environments (Segawa *et al.*, 2013) and even the Antarctic (Wang *et al.*, 2016). The use of antibiotics in therapy and livestock production has greatly promoted the development and spread of antibiotic resistance making impacted environments into large reservoirs of ARGs (Larsson *et al.*, 2018; Manaia *et al.*, 2018). Furthermore, the prevalence of mobile genetic elements, e.g. transposons, integrons and plasmids, could promote the horizontal gene transfer of ARGs to other bacteria including human pathogens, exacerbating the antimicrobial resistance issue (Gillings *et al.*, 2015; Ma *et al.*, 2017b).

Previously studies on antibiotic resistance mainly focused on clinical microorganisms to address the direct threat of emerging antibiotic resistant bacteria on public health. With the emergence of superbugs (multidrug resistant human pathogens) in medical treatment, both medical and public concern has increased. This has led to recognition that this is a problem that requires an interdisciplinary vision since it clearly involves not only human medicine but also agriculture and veterinary medicine as well as a range of environmental domains. The result has been formulation of the “One-Health” concept to engage scientists and practitioners across these disciplines to study the

problem and its remedies in a cross-disciplinary manner. Antibiotic resistance has been described as the "quintessential" One–Health issue (Robinson *et al.*, 2006). It involves antimicrobial resistance (AMR) in three main domains (Figure 1): Human health, animal agriculture and the environment. The United Nations through its agencies: the UNEP, the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organisation for Animal Health (OIE) work jointly to promote collective action to minimize the emergence and spread of AMR. As illustrated in Figure 1, soil is a central component in One-Health since it harbors a large natural resistome but also receives ARB and ARGs from both human and animal wastes which can be returned to humans through vegetable and animal products, through surface, ground and reclaimed water, and via aerosols. This is particularly important for soil science research because of: i) its central role as a reservoir, thus potential source for resistance traits, ii) the many routes of dissemination including to humans and iii) the large natural background of resistance which makes it more difficult to assess human-enhanced resistance and high risk AMR.

2 Detection of antibiotic resistance genes in the environment

One of the major needs to address the antibiotic resistance issue is improved AMR surveillance incorporating clinical, agricultural and environmental data at local, national and global levels (Hughes *et al.*, 2016). This has been a challenge for the environmental domain since a legacy of methods tuned to the environment has not been established. In the last decade, molecular methods to quantify ARGs in the environment and their distribution and propagation in wastewater and sludge, aquaculture, soil and atmosphere have been developed or improved. These are primarily of three types: PCR based amplification of ARGs, hybridization of DNA to ARG gene fragments and searching metagenomic sequence data for ARGs. The former includes the polymerase chain reaction (PCR), quantitative real-time PCR (qPCR), DNA microarray and DNA hybridization. The traditional target sequence amplification-based methods, PCR and qPCR, have been widely applied to detect and quantify ARGs in diverse environmental samples because of their high sensitivity for ARGs detection under low

abundance in natural environments. However, due to the limitations of primers available for ARGs amplification, PCR and qPCR approaches could only effectively detect well-studied ARGs. Besides, ARGs primers may cause biases and false negative/positive results, and only several ARGs could be detected in one set using traditional methods (Laht *et al.*, 2014; Titilawo *et al.*, 2015; Xiong *et al.*, 2015). In recent years, improvements have provided high-throughput (highly parallel) qPCR for detection of hundreds of ARGs in one run (Looft *et al.*, 2012). To further improve its efficiency and coverage, 294 primer sets for ARGs and some mobile genetic elements (MGEs) were developed (Zhu *et al.*, 2013) and validated by sequencing its amplicons (Johnson *et al.*, 2016). This was subsequently expanded to 384 primers (Wang *et al.*, 2016). Recently, the results from over 500 diverse samples were evaluated, and together with updated reference data and a new primers design tool, EcoFunPrimer, Primer set 2.0 for highly parallel qPCR was tested and reported (Stedtfeld *et al.*, 2018).

The rapid development of high-throughput sequencing (HTS) technology in the past decade has enabled sequence-based metagenomic analysis to be widely applied in diverse ecological, medical, engineering and physical studies (Kircher and Kelso, 2010), including in assaying ARB and ARGs in environmental samples (Zeng *et al.*, 2015; Ma *et al.*, 2016). HTS technique can rapidly generate large amounts of sequence data at a relatively low cost (Kircher and Kelso, 2010). Metagenomic analysis is a culture-independent molecular approach, which uses the generated metagenomic sequences to search, annotate and predict targeted genes. For annotation and detection of ARGs in environmental samples, the generated metagenomic sequences are filtered for quality control and then compared against a reference ARG database that contains sequences of the known ARGs. With the increasing concerns of ARB and ARGs in both medical treatment and environmental monitoring, several ARG databases have been constructed to facilitate the ARGs annotation, including ARDB (Liu and Pop, 2009), CARD (Jia *et al.*, 2017), ARG-ANNOT (Gupta *et al.*, 2014) and ResFinder (Zankari *et al.*, 2012). One structured database derived from combining ARBD and CARD was designed to search metagenomic data and classify the annotation results into 24 ARG types and 1208 subtypes (Yin *et al.*, 2018).

A variation of the above approach of searching for ARGs in metagenomic data is to first assemble the short-read ARG fragments to gain more reliability in gene identification. This can be done by gene-targeted assembly tools such as Xander (Wang *et al.*, 2015) or Mega GTA (Li *et al.*, 2017). One study reports this result in soils for 35 ARGs (Dunivin and Shade, 2018).

Challenges remain on improving the detection limit and identifying untargeted ARGs, which can be potentially-emerging, problematic ARGs. All of the above molecular methods are based on sequence information from known ARGs. None of these methods can detect cryptic ARGs. Further, many ARG sequences may be part of the natural resistome, code for functions that by themselves do not confer resistance, or may not be expressed.

The comparison of antimicrobial resistance among different environmental niches with culture-based clinical (pathogen) resistance is necessary for improving our knowledge on the evolution, spread and risk of ARGs in the environment. However, standard methods and criteria specifically designed for meeting this need in environmental samples is lacking. One challenge for use of culture-based methods for environmental samples is that > 99% of environmental microorganisms are not cultivable, and another is that pathogen recovery can be inefficient due to their injury or stress from being in the environment. Therefore, improvement of methods through combining molecular methods, perhaps informed by DNA sequence or biochemical markers from clinical or culture-based studies, with high-throughput sequencing will provide a comprehensive overview of antimicrobial resistance as well as specific areas of higher risk in diverse environmental samples. One example of that kind of method is epicPCR which can link the host and ARG without culturing in high throughput method (Hultman *et al*., 2018). Future attention should also be paid to tracking antimicrobial resistance from pollution sources versus natural environments to human-related environments, as important guidance for management of antimicrobial resistance risk.

3 Antibiotic resistance genes in wastewater and sludge

The major environmental sources of AMR, and especially those that are clinically relevant and

have the potential to reach humans are from i) urban wastewater treatment plants, ii) use in aquaculture, iii) use in animal agricultural for growth promotion and therapeutic use. Other sources, not discussed here, but should not be neglected, are antibiotic production and formulation factories and used as a 'pesticide' on some food crops. Furthermore, the use of antibiotics for treatment of companion animals, and transmission of ARB by wildlife, including birds, and the irrigation of crops with reclaimed wastewater, are sources to also be recognized (Fig. 1).

Domestic wastewater treatment plants, also known as urban wastewater treatment plants (UWTPs), also receive ARGS and ARBs from domestic and clinical sources but cannot remove them nor most antibiotics by the most commonly used technologies applied in the treatment of domestic effluents. Therefore, in spite of reducing the abundance of bacteria in the effluents, wastewater treatment, fulfilling the legal recommendations (e.g. Directive 91/271/EEC on Urban Waste Water Treatment and following amendments in the European Union), leads to the continuous release of ARB and ARGs into the environment (Vaz-Moreira *et al.*, 2014; Manaia *et al.*, 2016). Unfortunately, the risks posed by these high loads of ARBs and ARGs are still poorly understood (Czekalski *et al.*, 2014; Li *et al.*, 2016; Osinska *et al.*, 2016; Proia *et al.*, 2016; Li *et al.*, 2018).

The recognition of antibiotic resistance in waste water effluents has stimulated the study of occurrence and potential control of antibiotic resistance in UWTPs. Most abundant classes of ARGs were found in raw influents and final effluents, accumulated in activated sludge during secondary/biological treatment or reaching the UWTP surrounding environment (Karkman *et al.*, 2018). Not surprisingly, these genes are frequently detected in or statistically associated to bacterial genera and species known to be hosted transiently or permanently by humans (Narciso-da-Rocha *et al.*, 2014; Narciso-da-Rocha *et al.*, 2018). In addition, the presence of mobile genetic elements (MGEs), involved in horizontal gene transfer (HGT), particularly, plasmids and phages, as well as, genetic recombination elements in the wastewater metagenome, highlights the potential for the propagation of ARGs, within and across different environmental compartments (Dolejska and Papagiannitsis, 2018). Of note, is the fact that this core wastewater resistome (totality of resistance

genes) and mobilome (the totality of elements involved in HGT and genetic recombination) is continuously evolving by the rapid incorporation of genes emerging in clinical settings (Karkman *et al.*, 2018).

Advanced treatment options, such as chlorination, ozonation or UV, or combined disinfection approaches may reduce the environmental emission of ARGs from UWTPs (Dodd, 2012; Giannakis *et al.*, 2018). The reactivation and regrowth of bacteria after disinfection, particularly of fast growing bacteria, also may need attention in order to minimize the proliferation of ARB or ARGs after disinfection and during water storage (Becerra-Castro *et al*., 2016; Sousa *et al.*, 2017).

4 Antibiotic resistance genes in aquaculture

As a global source of fish and shellfish, the significance of aquaculture has increased remarkably in the last 50 years (Watts *et al.*, 2017), with the aquaculture production already surpasses the catch of the wild growing aquatic species. The term aquaculture can refer to a large spectrum of activities. The extensive aquaculture relies on native species which are helped by removing predators and competing species whereas in the intensive aquaculture the cultured species are more isolated from the environment and the food is supplied externally. On the other hand, the term covers everything between farming salmon offshore in the cold Atlantic water to tropical integrated aquaculture where excess animal feed and livestock manure are nutrients for the aquaculture. Therefore, it is not too surprising that there is no single picture of the antibiotic use in aquaculture.

Use of antibiotics in aquaculture is strictly regulated in Europe, Japan and North America. In those regions, the use of antibiotics is limited to therapeutic applications where only a limited number of antibiotics are approved. As an example, only five different antibiotics are authorized for use in aquaculture in the UK, compared to 13 different ones in China (Liu *et al.*, 2017). Some forms of aquaculture use antibiotics in a sustainable way, with the salmon production in Norway as probably the best example. Between 1987 and 2013 the antibiotic use in that production system was reduced by 99% concurrent with a 20-fold increase in the tonnage produced (Norwegian Ministry of Health

and Care Services 2015). The main factors behind such development are implementation of strict use of vaccines and hygienic requirements, which partly resulted from active scientific research in this field. There are obviously other than scientific and technological challenges involved since the uses of antibiotics in aquaculture varies tremendously even with the same fish species. The reason behind that difference is still not clear but it probably includes not only lack of vaccination, but also high fish density and sub-optimal fishing practices which includes at least underdeveloped hygiene and feeding with unknown components that might contain antibiotics or other agents causing the selection pressure for antibiotic resistance. Additionally, aquaculture systems integrating the wastes from livestock production are effective for nutrient cycling, but may have potential problems of disseminating antibiotic resistance (Cabello *et al.*, 2016).

5 Antibiotic resistance genes in soil

Of all the environments impacted by humans, soil undoubtedly contains the richest and most diverse populations of microorganisms (Thompson *et al.*, 2017) and DNA sequence (Rodriguez-R *et al.*, 2018). Soil bacteria carry antibiotic resistance genes that are also found in human clinical pathogens, and novel ones that are for the first time being discovered (Lau *et al.*, 2017). A key human activity of concern with respect to antimicrobial resistance that impacts the soil environment is the land application of fecal material, largely through agricultural practice (O'Connor *et al.*, 2005; Larney, 2011; Wang *et al.*, 2018). Organic amendments of animal (manures) or human (i.e. biosolids) origin are widely used as a valued source of nutrients and for crop production and organic matter for soil improvement. The use of antibiotics for prophylaxis or therapy in commercial food animal production will select for and enrich ARB in the digestive tract, and these bacteria will end up in the manure (Zhu *et al.*, 2013). Manure will also contain residues of antibiotics that have been excreted intact in the urine or feces (Pope *et al.*, 2009). The microbial and chemical composition of raw manures will vary with the commodity (i.e. poultry, swine, beef, dairy) and the medications used. Likewise, biosolids contain residues of antibiotics and other potential co-selective agents such as biocides

(Sabourin *et al.*, 2012). Treatment practices that reduce the abundance of ARB and destroy antibiotic residues will reduce soil loading rates of these contaminants and are therefore desirable (Lau *et al.*, 2017; Tien *et al.*, 2017).

Another increasingly important practice of concern with respect to potentiating antibiotic resistance in soils is irrigation with reclaimed wastewater (Christou *et al.*, 2017). Irrigation with nutrient-rich raw or poorly treated sewage is sometimes practiced in lower income countries (Thebo *et al.*, 2017). Turfgrass in green spaces in urban or peri-urban settings may also be irrigated with reclaimed wastewater (Wang *et al.*, 2014). Depending on the degree of treatment, reclaimed wastewater will contain ARB and residues of pharmaceuticals including antibiotics (Pan and Chu, 2018).

Soil contamination with metals and organic chemicals other than antibiotics that co-select for antibiotic resistance are of concern. Contamination of soil with copper and zinc from mining activities or the application of manure from animals that consume feed supplemented with the metals can co-select for genetic elements that confer resistance to both metals and antibiotics (Poole, 2017). Copper is also commonly spread on fruits and vegetables as a pesticide against bacterial and fungal plant pathogens. Interestingly, some herbicides decrease the sensitivity of Gram negative bacteria to some antibiotics, possibly through upregulating efflux (Kurenbach *et al.*, 2015). Likewise, there is a concern that the widespread use of fungicides in crop production will select for resistance that will end up eroding the efficacy of fungicides used to treat human fungal or yeast infections (Jensen, 2016). Fungicides are also entrained into soil through the application of biosolids (Chen *et al.*, 2013).

Given the chemical, biological and physical complexity of manures and biosolids it is extremely difficult to determine which specific agent or classes of agents interact with soil microorganisms to promote antibiotic resistance. Laboratory evidence suggests that in the presence of antibiotics, viruses (bacteriophage) disseminate antibiotic resistance genes more rapidly in manured soil than in the absence of antibiotics (Ross and Topp, 2015).

Soil can also affect the growth inhibition and selection pressure of antibiotics on the microbes

by sorption of the antibiotic reducing their bioavailability, which is determined by the chemical properties of the antibiotic and the soil matrix. In some cases soil reduces the growth inhibition of pathogens by certain antibiotics and for other antibiotics it does not (Chander *et al.*, 2005; Subbiah *et al.*, 2011). More recent mechanistic work shows that soils and their associated mineral and organic constituents affects the bioavailability of tetracycline as measured by a bioreporter linked to ARG expression (Chen *et al.*, 2017). Antibiotic residues that are sorbed on clay mineral surfaces turned on the bioreporter suggesting that they would be able to provide selective pressure for antimicrobial resistance (Zhang *et al.*, 2018).

6 Antibiotic resistance genes in the atmosphere

Due to its emerging threat to public health, the existence of ARGs in the atmosphere, especially urban air, has given rise to more attention. Comprehensive knowledge of the types and abundance of ARGs in the air and whether they are associated with potential pathogens can provide new guidance for assessment of air quality.

The relative abundance profiles of ARGs are spatially and temporally distributed in ambient air of various cities. On heavy air pollution days, the increase of airborne particles is conductive to suspending of microbes by providing more adhesion sites (Hu *et al.*, 2018). There was a higher richness with 64 ARG types in bacterial biota in Beijing smog than in other environments, such as pharmaceutically polluted environments, wastewater/sludge, animals and other terrestrial sources (Pal *et al.*, 2016). These results indicate that airborne transmission plays a crucial role in the global environmental distribution and exposure of antimicrobial resistance compared with other routes. Evidence support that such a risk is attributed to diverse antibiotic usage in different cities. In rural settings, airborne particulate matter promoted the transmission of microbial biota harboring antimicrobial resistance genes downwind of cattle feedlots (McEachran *et al.*, 2015). The dispersal patterns of particulate matter-borne ARGs were also impacted by physicochemical factors, meteorological parameters and bacterial communities (Hu *et al.*, 2018). Pathogens in clinical ambient

air may be resistant to multiple drugs, threating inhabitants as well as people outside hospitals via airborne spread (Huang *et al.*, 2012).

Unfortunately, knowledge on airborne ARGs in geographically, culturally, and economically different areas on a global scale is lacking. Their health impact is still unknown. On the other hand, considerable attention is paid on particulate matter mass concentration, e.g. PM 2.5, while biological parameters such as ARGs or ARB are yet to be adequately evaluated (Li *et al*., 2018).

7 Transmission of antibiotic resistance genes in the environment

Two major factors determine the fate of ARGs in the environment: dispersal mechanisms and selection. The dissemination of resistance genes is attributed to physical and biological forces throughout various environments. Physical forces drive the dispersal of ARGs, such as wind and waters, including erosion and leaching, but so do wild animals, e.g. birds, deer, raccoons. Major routes of human exposures are from contaminated foods, e.g. vegetables with exposure to manures or reclaimed wastewater, or meats contaminated with fecal waste from slaughter (Fig. 1). Less likely sources are from drinking water, which is highly source dependent, and air. Pets can also be a source and a recipient from their owner, and thereby cycle the AMR (Fig. 1). Proximity to human activities provides an enriched source of ARGs for potential dispersal. For example, human proximity is closely related to the antibiotic resistance profiles of the gut bacteria of wild mammals, whose habitats can be affected by antibiotics (Allen *et al.*, 2010).

The selection pressure exerted on environmental bacteria can be of two types: selection for the microbes that are carrying ARGs that is independent of the resistance trait, e.g. for particular carbon sources, faster growth rate, resistances to stressors like pH; and selection for ARG trait which can be the antibiotic itself or co-selection for resistances that are genetically linked to another (or multiple) ARGs. The type of selective agent (chemical speciation and concentrations), co-exposure to other selective agents (exposure period) and under what environmental conditions are essential for differential bacterial growth (Baquero *et al.*, 2009; Larsson *et al.*, 2018). An overview of the

299 characteristics, variability and abundance of ARGs and mobile genetic elements, and their
300 transmission efficiencies in various circumstances is necessary to better differentiate ambient from
301 anthropogenic sources of antibiotics and ARGs in the environment (Hunter *et al.*, 2008; Zhu *et al.*,
302 2017; Larsson *et al.*, 2018). Such transfer and selection patterns of genes and bacteria are important
303 guides to distinguish the most urgent exposure and risk reduction practices.
304
305 **8 Conclusions and One Health perspective**
306 Humans profoundly impact the environment, in particular through agricultural practice. There
307 is a need to better understand what specific practices represent what risk with respect to AMR in soil,
308 water, air, and human health. This needs to be done considering the realities of how agriculture and
309 aquaculture are undertaken in high, middle and lower-income settings. There is also a need to better
310 understand how the fate and impacts of microbial and chemical contaminants that reach soil and water
311 vary with climate, particularly extremes of temperature and rainfall.
312 Importantly, changes in food animal production practices that reduce antimicrobial use will
313 reduce selection for and transmission of antibiotic-resistant bacteria. One practice that has long been
314 questioned is the use of sub-therapeutic doses for growth promotion in animal production. It has been
315 widely practiced because it does speed time to market and hence lower cost of food, but it has been
316 targeted as a non-essential practice that promotes AMR. Progress is being made though as this practice
317 has been banned in the EU since January 2006 and in the U.S. since January 2017. This could only
318 be accomplished in concert with changes in production systems that minimize disease and maintain
319 farmer profitability. Currently vaccination is an economically viable practice especially with high
320 value species (e.g. salmon) in some developed countries. Development of alternatives to antibiotics,
321 like vaccines, is needed but they must be cost competitive to have an impact on antibiotic use. This
322 is an important area for research.
323 The relevance of ARB and ARGs as environmental contaminants is now accepted but there still
324 exist some key priority issues. There is currently limited evidence that permit an objective and

quantitative assessment of the human health risks posed by the occurrence of ARB in the environment. For this reason, it is difficult to define threshold values for the maximum admissible levels of ARB and ARGs in treated wastewater, in sludge and manure to be used in agriculture. Increasing water scarcity and soil degradation will drive increased need for water reuse, and beneficial reuse of municipal sludges and manures, likely increasing inputs of ARGs and ARBs into soil. The movement of people and food between higher and lower income countries will contribute to the global transmission of resistance from regions with poor sanitary conditions. Improving water sanitation and hygiene in lower income countries should be a global priority. Overall, adequate risk assessment frameworks, policy development and implementation, definition of circular economy good practices and ensuring adequate sanitation are priorities to reduce antibiotic resistance burden and global transmission (Pruden *et al*., 2013).

The One Health framework was developed in recognition of the inter-domain system in which antibiotic resistance resides and circulates (Fig. 1). Among the three domains, human health is the spotlight, due to multidrug-resistance genes have been prevalent in several important pathogens. To achieve the goal of One Health, veterinarians, physicians, food safety professionals, wastewater treatment plant operators, environmental experts and regulators are obliged to work together. At a policy level, all countries were asked to formulate a "National Action Plan" to combat antimicrobial resistant appropriate for their country (WHO, 2017). Many have done so, but the challenge is now to effectively implement and monitor these plans, and to validate the efficacy of practices intended to reduce the development and spread of AMR. Thus the overriding importance of undertaking surveillance is across the One Health Framework.

Acknowledgments

F. Wang's research is funded by the National Natural Science Foundation of China (21677149), the Outstanding Youth Fund of Natural Science Foundation of Jiangsu, China (BK20150050), the Innovative Project of the Chinese Academy of Sciences (ISSASIP1616). J. M. Tiedje's research is

funded by, and the Center for Health Impacts of Agriculture (CHIA) of Michigan State University. E. Topp's research is funded by Agriculture and Agri-Food Canada, the Canadian Genomics Research Development Initiative (GRDI-AMR), and the Canadian Institute for Health Research (CIHR) through the Joint Programming Initiative on Antimicrobial resistance (JPIAMR). C. M. Manaia acknowledges the National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UID/Multi/50016/2013. M. Virta's research is funded by Academy of Finland and JPI-Water.

References

Allen H K, Donato J, Wang H H, Cloud-Hansen K A, Davies J, Handelsman J. 2010. Call of the wild: Antibiotic resistance genes in natural environments. *Nat Rev Microbiol.* **8**: 251-259.

Baquero F, Alvarez-Ortega C, Martinez J L. 2009. Ecology and evolution of antibiotic resistance. *Environ Microbiol Rep.* **1**: 469-476.

Becerra-Castro C, Macedo G, Silva A M T, Manaia C M, Nunes O C. 2016. Proteobacteria become predominant during regrowth after water disinfection. *Sci Total Environ.* 573: 313-323.

Berendonk T U, Manaia C M, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Burgmann H, Sorum H, Norstrom M, Pons M N, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez J L. 2015. Tackling antibiotic resistance: The environmental framework. *Nat Rev Microbiol.* **13**: 310-317.

Cabello F C, Godfrey H P, Buschmann A H, Dölz H J. 2016. Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. *Lancet Infec Dis.* **16**: e127-e133.

Chander Y, Kumar K, Goyal S M, Gupta S C. 2005. Antibacterial activity of soil-bound antibiotics. *J Environ Qual.* **34**: 1952-1957.

Chen Z F, Ying G G, Ma Y B, Lai H J, Chen F, Pan C G. 2013. Typical azole biocides in biosolid-amended soils and plants following biosolid applications. *J Agr Food Chem.* **61**: 6198-6206.

Chen Z, Zhang W, Wang G, Zhang Y, Gao Y, Boyd S A, Teppen B J, Tiedje J M, Zhu D, Li H. 2017.

377 Bioavailability of soil-sorbed tetracycline to escherichia coli under unsaturated conditions.
378 *Environ Sci Technol.* **51**: 6165-6173.
379 Christou A, Agüera A, Bayona J M, Cytryn E, Fotopoulos V, Lambropoulou D, Manaia C M, Michael
380 C, Revitt M, Schröder P, Fatta-Kassinos D. 2017. The potential implications of reclaimed
381 wastewater reuse for irrigation on the agricultural environment: The knowns and unknowns
382 of the fate of antibiotics and antibiotic resistant bacteria and resistance genes - a review. *Water*
383 *Res.* **123**: 448-467.
384 Czekalski N, Gascón Díez E, Bürgmann H. 2014. Wastewater as a point source of antibiotic-
385 resistance genes in the sediment of a freshwater lake. *ISME J.* **8**: 1381-1390.
386 Dodd M C. 2012. Potential impacts of disinfection processes on elimination and deactivation of
387 antibiotic resistance genes during water and wastewater treatment. *J Environ Monitor.* **14**:
388 1754-1771.
389 Dolejska M, Papagiannitsis C C. 2018. Plasmid-mediated resistance is going wild. *Plasmid.* **99**: 99-
390 111.
391 Dunivin T K, Shade A. 2018. Community structure explains antibiotic resistance gene dynamics over
392 a temperature gradient in soil. *FEMS Microbiol Ecol.* **94**: 1-9.
393 Garbisu C, Garaiyurrebaso O, Lanzen A, Alvarez-Rodriguez I, Arana L, Blanco F, Smalla K,
394 Grohmann E, Alkorta I. 2018. Mobile genetic elements and antibiotic resistance in mine soil
395 amended with organic wastes. *Sci Total Environ.* **621**: 725-733.
396 Giannakis S, Le T M, Entenza J M, Pulgarin C. 2018. Solar photo-fenton disinfection of 11 antibiotic-
397 resistant bacteria (ARB) and elimination of representative ar genes. Evidence that antibiotic
398 resistance does not imply resistance to oxidative treatment. *Water Res.* **143**: 334-345.
399 Gillings M R, Gaze W H, Pruden A, Smalla K, Tiedje J M, Zhu Y G. 2015. Using the class 1 integron-
400 integrase gene as a proxy for anthropogenic pollution. *ISME J.* **9**: 1269-1279.
401 Gupta S K, Padmanabhan B R, Diene S M, Lopez-Rojas R, Kempf M, Landraud L, Rolain J M. 2014.
402 Arg-annot, a new bioinformatic tool to discover antibiotic resistance genes in bacterial

403 genomes. *Antimicrob Agents Ch.* **58**: 212-220.
404 Hu J, Zhao F, Zhang X-X, Li K, Li C, Ye L, Li M. 2018. Metagenomic profiling of args in airborne
405 particulate matters during a severe smog event. *Sci Total Environ.* **615**: 1332-1340.
406 Huang X Z, Frye J G, Chahine M A, Glenn L M, Ake J A, Su W, Nikolich M P, Lesho E P. 2012.
407 Characteristics of plasmids in multi-drug-resistant enterobacteriaceae isolated during
408 prospective surveillance of a newly opened hospital in iraq. *Plos One.* **7**: 1-8.
409 Hughes M J, Tiedje M J, Bell B, Call D, Cassell G, Crawford C, Granger L, Miller A. 2016. *AMR*
410 *Steering Commitee Meeting Summary, American Society for Microbiology, Washington DC.*
411 Hultman J, Tamminen, M, Pärnänen, K, Cairns, J, Karkman, A,Virta, M. 2018. Host range of
412 antibiotic resistance genes in wastewater treatment plant influent and effluent. *FEMS Microb*
413 *Ecol.* **94;**: fiy038.
414 Hunter P R, Wilkinson D C, Catling L A, Barker G C. 2008. Meta-analysis of experimental data
415 concerning antimicrobial resistance gene transfer rates during conjugation. *Appl Environ*
416 *Microb.* **74**: 6085-6090.
417 Jensen R H. 2016. Resistance in human pathogenic yeasts and filamentous fungi: Prevalence,
418 underlying molecular mechanisms and link to the use of antifungals in humans and the
419 environment. *Dan Med J.* **63**: 1-11.
420 Jia B F, Raphenya A R, Alcock B, Waglechner N, Guo P Y, Tsang K K, Lago B A, Dave B M, Pereira
421 S, Sharma A N, Doshi S, Courtot M, Lo R, Williams L E, Frye J G, Elsayegh T, Sardar D,
422 Westman E L, Pawlowski A C, Johnson T A, Brinkman F S L, Wright G D, McArthur A G.
423 2017. Card 2017: Expansion and model-centric curation of the comprehensive antibiotic
424 resistance database. *Nucleic Acids Res.* **45**: D566-D573.
425 Johnson T A, Stedtfeld R D, Wang Q, Cole J R, Hashsham S A, Looft T, Zhu Y-G, Tiedje J M. 2016.
426 Clusters of antibiotic resistance genes enriched together stay together in swine agriculture.
427 *Mbio.* **7**: e02214-e02215.
428 Karkman A, Do T T, Walsh F, Virta M P J. 2018. Antibiotic-resistance genes in waste water. *Trends*

Microbiol. **26**: 220-228.

Kircher M, Kelso J. 2010. High-throughput DNA sequencing - concepts and limitations. *Bioessays.* **32**: 524-536.

Kurenbach B, Marjoshi D, Amabile-Cuevas C F, Ferguson G C, Godsoe W, Gibson P, Heinemann J A. 2015. Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in escherichia coli and salmonella enterica serovar typhimurium. *Mbio.* **6**: 1-9.

Laht M, Karkman A, Voolaid V, Ritz C, Tenson T, Virta M, Kisand V. 2014. Abundances of tetracycline, sulphonamide and beta-lactam antibiotic resistance genes in conventional wastewater treatment plants (wwtps) with different waste load. *PLoS One.* **9**: 1-8.

Larney F J, X. Hao, and E. Topp. 2011 Soil management: Building a sustainable base for agriculture American Society for Agronomy, Madison WI.

Larsson D G J, Andremont A, Bengtsson-Palme J, Brandt K K, Husman A M d R, Fagerstedt P, Fick J, Flach C F, Gaze W H, Kuroda M, Kvint K, Laxminarayan R, Manaia C M, Nielsen K M, Plant L, Ploy M C, Segovia C, Simonet P, Smalla K, Snape J, Topp E, van Hengel A J, Verner-Jeffreys D W, Virta M P J, Wellington E M, Wernersson A-S. 2018. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. *Environ Int.* **117**: 132-138.

Lau C H F, Li B, Zhang T, Tien Y C, Scott A, Murray R, Sabourin L, Lapen D R, Duenk P, Topp E. 2017. Impact of pre-application treatment on municipal sludge composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest. *Sci Total Environ.* **587**: 214-222.

Lau C H F, Van Engelen K, Gordon S, Renaud J, Topp E. 2017. Novel antibiotic resistance determinants from agricultural soil exposed to antibiotics widely used in human medicine and animal farming. *Appl Environ Microbiol.* **83**: 1-18.

Leonard A F C, Zhang L, Balfour A, Garside R, Gaze W H. 2015. Human recreational exposure to

antibiotic resistant bacteria in coastal bathing waters. *Environ Int*. 82: 92-100.

Li A, Chen L, Zhang Y, Tao Y, Xie H, Li S, Sun W, Pan J, He Z, Mai C, Fan Y, Xian H, Zhang Z, Wen D. 2018. Occurrence and distribution of antibiotic resistance genes in the sediments of drinking water sources, urban rivers, and coastal areas in zhuhai, china. *Environ Sci Pollut R.* **25**: 26209-26217.

Li C, Lu J, Liu J, Zhang G, Tong Y, Ma N. 2016. Exploring the correlations between antibiotics and antibiotic resistance genes in the wastewater treatment plants of hospitals in Xinjiang, China. *Enviro Sci Pollut R.* **23**: 15111-15121.

Li D, Huang Y, Leung C M, Luo R, Ting H F, Lam T W. 2017. Megagta: A sensitive and accurate metagenomic gene-targeted assembler using iterative de bruijn graphs. *BMC Bioinformatics.* **18**: 408-417.

Li L G, Yin X, Zhang T. 2018. Tracking antibiotic resistance gene pollution from different sources using machine-learning classification. *Mbio.* **6**: 93-93.

Liu B, Pop M. 2009. Ardb-antibiotic resistance genes database. *Nucleic Acids Res.* **37**: D443-D447.

Liu X, Steele J C, Meng X Z. 2017. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environ Pollut.* **223**: 161-169.

Looft T, T. A. Johnson, H.K. Allen, D.O. Bayles, D.P. Alt, R.D. Stedtfeld, W.J. Sul, T.M. Stedtfeld, B. Chai B, J. R. Cole, S.A. Hashsham, J.M.Tiedje and T.B. Stanton. 2012. In-feed antibiotic effects on the swine intestinal microbiome. *P Natl Acad Sci USA.* 109(5): 1691-1696.

Ma L P, Li A D, Yin X L, Zhang T. 2017b. The prevalence of integrons as the carrier of antibiotic resistance genes in natural and man-made environments. *Environ Sci Technol.* **51**: 5721-5728.

Ma L P, Li B, Jiang X T, Wang Y L, Xia Y, Li A D, Zhang T. 2017a. Catalogue of antibiotic resistome and host-tracking in drinking water deciphered by a large scale survey. *Mbio.* **5**: 154-166.

Ma L P, Xia Y, Li B, Yang Y, Li L G, Tiedje J M, Zhang T. 2016. Metagenomic assembly reveals hosts of antibiotic resistance genes and the shared resistome in pig, chicken, and human feces. *Environ Sci Technol.* **50**: 420-427.

481 Manaia C M, Macedo G, Fatta-Kassinos D, Nunes O C. 2016. Antibiotic resistance in urban aquatic
482 environments: Can it be controlled? *Appl Microbiol and Biot.* **100**: 1543-1557.
483 Manaia C M, Rocha J, Scaccia N, Marano R, Radu E, Biancullo F, Cerqueira F, Fortunato G,
484 Iakovides I C, Zammit I, Kampouris I, Vaz-Moreira I, Nunes O C. 2018. Antibiotic resistance
485 in wastewater treatment plants: Tackling the black box. *Environ Int.* **115**: 312-324.
486 McEachran A D, Blackwell B R, Hanson J D, Wooten K J, Mayer G D, Cox S B, Smith P N. 2015.
487 Antibiotics, bacteria, and antibiotic resistance genes: Aerial transport from cattle feed yards
488 via particulate matter. *Environ Health Persp.* **123**: 337-343.
489 Muziasari W I, Pitkanen L K, Sorum H, Stedtfeld R D, Tiedje J M, Virta M. 2017. The resistome of
490 farmed fish feces contributes to the enrichment of antibiotic resistance genes in sediments
491 below baltic sea fish farms. *Front Microbiol.* **8:** 1-10.
492 Narciso-da-Rocha C, Rocha J, Vaz-Moreira I, Lira F, Tamames J, Henriques I, Martinez J L, Manaia
493 C M. 2018. Bacterial lineages putatively associated with the dissemination of antibiotic
494 resistance genes in a full-scale urban wastewater treatment plant. *Environ Int.* **118**: 179-188.
495 Narciso-da-Rocha C, Varela A, Schwartz T, Nunes O, Manaia C. 2014. Blatem and vana as indicator
496 genes of antibiotic resistance contamination in a hospital-urban wastewater treatment plant
497 system. *J Glob Antimicrob Re*. **2**:309-315.
498 O'Connor G A, Elliott H A, Basta N T, Bastian R K, Pierzynski G M, Sims R C, Smith J E. 2005.
499 Sustainable land application: An overview. *J Environ Qual.* **34**: 7-17.
500 Osinska A, Harnisz M, Korzeniewska E. 2016. Prevalence of plasmid-mediated multidrug resistance
501 determinants in fluoroquinolone-resistant bacteria isolated from sewage and surface water.
502 *Environ Sci Pollut R.* **23**: 10818-10831.
503 Pal C, Bengtsson-Palme J, Kristiansson E, Larsson D G J. 2016. The structure and diversity of human,
504 animal and environmental resistomes. *Mbio.* **4**: 54-69.
505 Pan M, Chu L M. 2018. Occurrence of antibiotics and antibiotic resistance genes in soils from
506 wastewater irrigation areas in the pearl river delta region, southern china. *Sci Total Environ.*

507 **624**: 145-152.
508 Poole K. 2017. At the nexus of antibiotics and metals: The impact of cu and zn on antibiotic activity
509 and resistance. *Trends Microbiol.* **25**: 820-832.
510 Pope L, Boxall A B A, Corsing C, Halling-Sorensen B, Tait A, Topp E. 2009. Veterinary medicines
511 in the environment. SETAC, Pensacola, FL.
512 Proia L, von Schiller D, Sànchez-Melsió A, Sabater S, Borrego C M, Rodríguez-Mozaz S, Balcázar
513 J L. 2016. Occurrence and persistence of antibiotic resistance genes in river biofilms after
514 wastewater inputs in small rivers. *Environ Pollut.* **210**: 121-128.
515 Pruden A, Larsson D G J, Amézquita A, Collignon P, Brandt Kristian K, Graham David W, Lazorchak
516 James M, Suzuki S, Silley P, Snape Jason R, Topp E, Zhang T, Zhu Y G. 2013. Management
517 options for reducing the release of antibiotics and antibiotic resistance genes to the
518 environment. *Environmental Health Perspectives*. **121**: 878-885.
519 Robinson D A, Sutcliffe J A, Tewodros W, Manoharan A, Bessen D E. 2006. Evolution and global
520 dissemination of macrolide-resistant group a streptococci. *Antimicrob Agents Ch.* **50**: 2903-
521 2911.
522 Rodriguez-R L M, Gunturu S, Tiedje J M, Cole J R, Konstantinidis K T. 2018. Nonpareil 3: Fast
523 estimation of metagenomic coverage and sequence diversity. *Msystems.* **3:** 1-9.
524 Ross J, Topp E. 2015. Abundance of antibiotic resistance genes in bacteriophage following soil
525 fertilization with dairy manure or municipal biosolids, and evidence for potential transduction.
526 *Appl Environ Microbiol.* **81**: 7905-7913.
527 Sabourin L, Duenk P, Bonte-Gelok S, Payne M, Lapen D R, Topp E. 2012. Uptake of pharmaceuticals,
528 hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. *Sci*
529 *Total Environ.* **431**: 233-236.
530 Segawa T, Takeuchi N, Rivera A, Yamada A, Yoshimura Y, Barcaza G, Shinbori K, Motoyama H,
531 Kohshima S, Ushida K. 2013. Distribution of antibiotic resistance genes in glacier
532 environments. *Env Microbiol Rep.* **5**: 127-134.

533 Sousa J M, Macedo G, Pedrosa M, Becerra-Castro C, Castro-Silva S, Pereira M F R, Silva A M T,
534 Nunes O C, Manaia C M. 2017. Ozonation and uv254nm radiation for the removal of
535 microorganisms and antibiotic resistance genes from urban wastewater. *J Hazard Mater.* **323**:
536 434-441.
537 Stedtfeld R D, Guo X, Stedtfeld T M, Sheng H, Williams M R, Hauschild K, Gunturu S, Tift L, Wang
538 F, Howe A, Chai B, Yin D, Cole J R, Tiedje J M, Hashsham S A. 2018. Primer set 2.0 for
539 highly parallel qpcr array targeting antibiotic resistance genes and mobile genetic elements.
540 *FEMS Microbiol Ecol.* **94**: 1-9.
541 Subbiah M, Mitchell S M, Ullman J L, Call D R. 2011. Beta-lactams and florfenicol antibiotics remain
542 bioactive in soils while ciprofloxacin, neomycin, and tetracycline are neutralized. *Appl*
543 *Environ Microbiol.* **77**: 7255-7260.
544 Thebo A L, Drechsel P, Lambin E F, Nelson K L. 2017. A global, spatially-explicit assessment of
545 irrigated croplands influenced by urban wastewater flows. *Environ Res Lett.* **12**: 1-12.
546 Thompson L R, Sanders J G, McDonald D, Amir A, Ladau J, Locey K J, Prill R J, Tripathi A, Gibbons
547 S M, Ackermann G, Navas-Molina J A, Janssen S, Kopylova E, Vázquez-Baeza Y, González
548 A, Morton J T, Mirarab S, Zech Xu Z, Jiang L, Haroon M F, Kanbar J, Zhu Q, Jin Song S,
549 Kosciolek T, Bokulich N A, Lefler J, Brislawn C J, Humphrey G, Owens S M, Hampton-
550 Marcell J, Berg-Lyons D, McKenzie V, Fierer N, Fuhrman J A, Clauset A, Stevens R L, Shade
551 A, Pollard K S, Goodwin K D, Jansson J K, Gilbert J A, Knight R, The Earth Microbiome
552 Project C. 2017. A communal catalogue reveals earth's multiscale microbial diversity. *Nature.*
553 **551**: 457-477.
554 Tien Y C, Li B, Zhang T, Scott A, Murray R, Sabourin L, Marti R, Topp E. 2017. Impact of dairy
555 manure pre-application treatment on manure composition, soil dynamics of antibiotic
556 resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest. *Sci*
557 *Total Environ.* **581–582**: 32-39.
558 Titilawo Y, Obi L, Okoh A. 2015. Antimicrobial resistance determinants of escherichia coli isolates

559 recovered from some rivers in osun state, south-western nigeria: Implications for public health.
560 *Sci Total Environ.* **523**: 82-94.
561 UNEP. 2017. Antimicrobial resistance from environmental pollution among biggest emerging health
562 threats, says UN Environment. https://www.unenvironment.org/news-and-stories/press-
563 release/antimicrobial-resistance-environmental-pollution-among-biggest.
564 Vaz-Moreira I, Nunes O C, Manaia C M. 2014. Bacterial diversity and antibiotic resistance in water
565 habitats: Searching the links with the human microbiome. *FEMS Microbiol Rev.* **38**: 761-778.
566 Wang F H, Qiao M, Su J Q, Chen Z, Zhou X, Zhu Y G. 2014. High throughput profiling of antibiotic
567 resistance genes in urban park soils with reclaimed water irrigation. *Environ Sci Technol.* **48**:
568 9079-9085.
569 Wang F, Stedtfeld R D, Kim O S, Chai B, Yang L, Stedtfeld T M, Hong S G, Kim D, Lim H S,
570 Hashsham S A, Tiedje J M, Sul W J. 2016. Influence of soil characteristics and proximity to
571 antarctic research stations on abundance of antibiotic resistance genes in soils. *Environ Sci*
572 *Technol.* **50**: 12621-12629.
573 Wang F, Xu M, Stedtfeld R D, Sheng H, Fan J, Liu M, Chai B, de Carvalho T S, Li H, Li Z, Hashsham
574 S A, Tiedje J M. 2018. Long-term effect of different fertilization and cropping systems on the
575 soil antibiotic resistome. *Environ Sci Technol.* **52**: 13037-13046.
576 Wang Q, Fish J A, Gilman M, Sun Y, Brown C T, Tiedje J M, Cole J R. 2015. Xander: Employing a
577 novel method for efficient gene-targeted metagenomic assembly. *Mbio.* **3**: 32-45.
578 Watts E J, Schreier J H, Lanska L, Hale S M. 2017. The rising tide of antimicrobial resistance in
579 aquaculture: Sources, sinks and solutions. *Mar Drugs.* **15**: 1-16.
580 WHO. 2017. WHO supports steps to develop the country's National Action Plan on Antimicrobial
581 Resistance. https://afro.who.int/news/who-supports-steps-develop-countrys-national-action-
582 plan-antimicrobial-resistance.
583 Xiong W, Sun Y, Ding X, Wang M, Zeng Z. 2015. Selective pressure of antibiotics on args and
584 bacterial communities in manure-polluted freshwater-sediment microcosms. *Front Microbiol.*

585 **6**: 194-194.
586 Yin X L, Jiang X T, Chai B L, Li L G, Yang Y, Cole J R, Tiedje J M, Zhang T. 2018. Args-oap v2.0
587 with an expanded sarg database and hidden markov models for enhancement characterization
588 and quantification of antibiotic resistance genes in environmental metagenomes.
589 *Bioinformatics.* **34**: 2263-2270.
590 Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup F M, Larsen M
591 V. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemoth.* **67**:
592 2640-2644.
593 Zeng G, Wu H, Liang J, Guo S, Huang L, Xu P, Liu Y, Yuan Y, He X, He Y. 2015. Efficiency of
594 biochar and compost (or composting) combined amendments for reducing Cd, Cu, Zn and Pb
595 bioavailability, mobility and ecological risk in wetland soil. *RSC Adv.* **5**: 34541-34548.
596 Zhang Y, Boyd S A, Teppen B J, Tiedje J M, Zhang W, Zhu D, Li H. 2018. Bioavailability of
597 tetracycline to antibiotic resistant escherichia coli in water-clay systems. *Environ Pollut.* **243**:
598 1078-1086.
599 Zhu Y G, Johnson T A, Su J Q, Qiao M, Guo G X, Stedtfeld R D, Hashsham S A, Tiedje J M. 2013.
600 Diverse and abundant antibiotic resistance genes in chinese swine farms. *P Natl Acad Sci USA.*
601 **110**: 3435-3440.
602 Zhu Y G, Zhao Y, Li B, Huang C L, Zhang S Y, Yu S, Chen Y S, Zhang T, Gillings M R, Su J Q. 2017.
603 Continental-scale pollution of estuaries with antibiotic resistance genes. *Nat Microbiol*. **2**:
604 16270-16277.

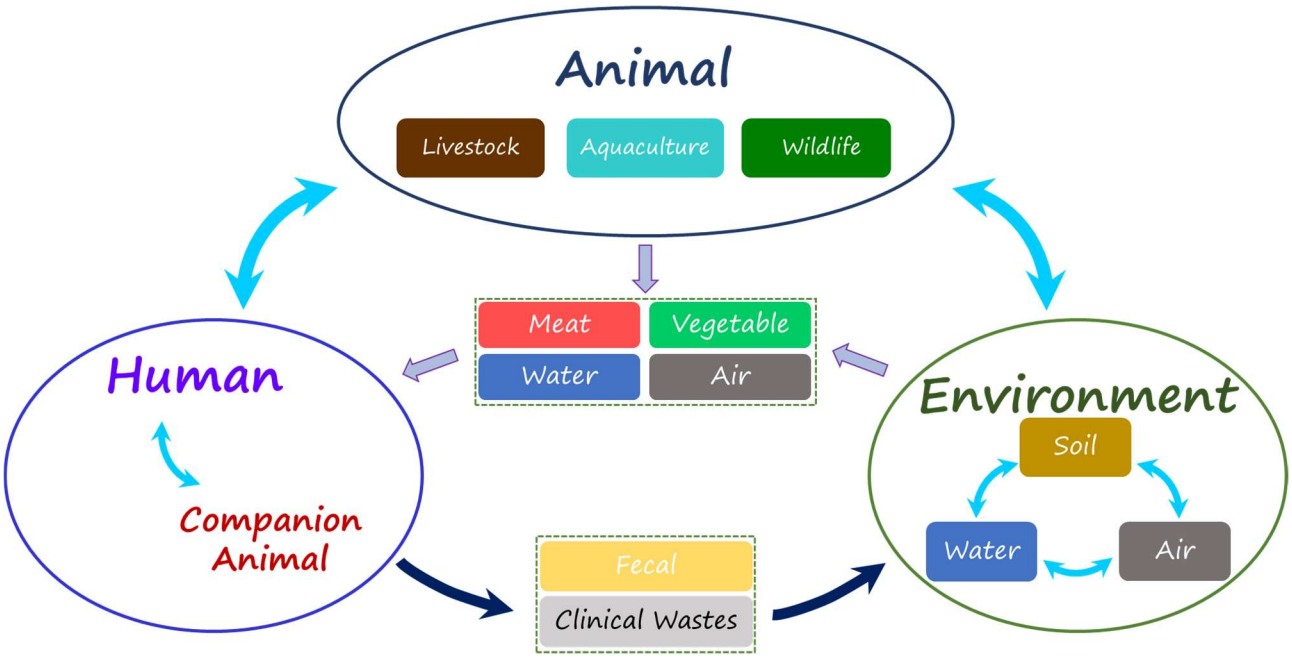

Fig. 1 The transmission of antibiotic resistance in the One Health framework with Human Health, Animals and Environment as the major domains through which antibiotic resistance genes and microbes reside, amplify and spread. Most arrows are in both directions reflecting these pathways, and the cyclic nature of the problem. Human exposure can be directly from animals and from food, water and air, but humans can also spread resistance to those domains, and especially via fecal wastes. Companion animals are grouped with humans because of higher potential for ARG exposure and bidirectional transfer.